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# Characterization of the mig-10(mp0920) mutant and its effects on migration in Caenorhabditis elegans

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# Characterization of the *mig-10(mp0920)* mutant and its effects on migration in *Caenorhabditis elegans*

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Submitted by:

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Submitted to:

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## Abstract

MIG-10 is a protein that is required for the migration of certain neurons and outgrowth of the excretory cell process during development in *Caenorhabditis elegans*. The *mig-10 (mp0920)* allele was characterized by sequencing the coding region of the gene. Mutant animals were also characterized by measuring the migration distances of neurons and the excretory cell process length. Characterization of a novel missense allele would lead to a better understanding of the MIG-10 protein domains.

## Acknowledgements

I would like to thank the Biology and Biotechnology Department at Worcester Polytechnic Institute for sponsoring this project, as well as my advisors, Professor Elizabeth Ryder and Professor Michael Buckholt for their knowledge, patience and assistance. I would also like to thank Jo-Ellen J. Sullivan and An T. Zacharia for their previous work leading to the isolation of the *mig-10(mp0920)* mutation.

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# Introduction

## 1. Nervous System Development: Neuron Migration and Axon Outgrowth

Proper nervous system development is critical for health and functionality of an organism. The development of the nervous system is a complex process including many signaling molecules as well as transduction pathways. In order for proper nervous system function to occur, connections between varying neuronal cell types must be formed (Bear et al., 2001). This is done by neurons first migrating to their specific location in the organism, and then sending out axons to their specific destination in the organism. The axon uses a growth cone to navigate its way throughout the organism, and the growth cone uses flat membrane sheets called *lamellipodia* which navigate through the organism and use thin spikes called *filopodia* to constantly probe the environment (Figure 1.). As the filopodia extend out of the lamellipodia and advance forward, lamellipodia sheets extend forward, advancing the movement of the growth cone, and the growth of the axon (Sanes et al., 2006).

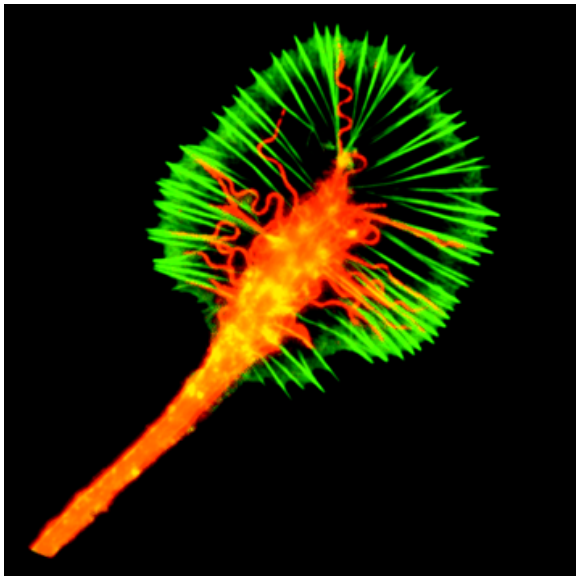

**Figure 1:** Growth cone present at the end of the axon, containing fingerlike filopodia (green stain) which extend from the growth cone (stained red). (*Picture taken from Goodwin, 2008)*

As the growth cone navigates through the organism during the outgrowth of the axon, it uses receptors to recognize specific guidance cues which are used to guide migration. These guidance cues are released into the extracellular matrix and can be either attractive or repulsive to the growth cone. Once the guidance cue is sensed by receptors on the growth cone it sends a signal to the rest of the axon indicating the correct orientation for its migration (Bear et al. 2001).

In this study the organism that was used in order to observe axon and cell migration as well as development was *C. elegans.* Mutations in the *mig-10* gene have been found to affect cell migration as well as axon guidance. The *mig*-10 gene has a human homolog, *lamellipodin* (Quinn and Wadsworth, 2008). Understanding the effects of this gene on worms will lead to the understanding of homologous genes in humans.

## 2. *Caenorhabditis elegans*

*Caenorhabditis elegans*, also known as *C. elegans*, is a non-parasitic soil-dwelling nematode which was introduced to the world of developmental biology and neurology research in 1963 by Sydney Brenner. These worms are commonly used as model systems in research laboratories due to their small size (about 1mm in length) and easy maintenance. *C. elegans* feed on bacteria such as *E. coli* and can live in large colonies (up to 10,000 worms/Petri dish). The genetic make-up of *C. elegans* includes six pairs of chromosomes, five pairs of autosomes and a pair of sex chromosomes. If a worm contains one X in the sixth chromosome the animal is a hermaphrodite and if it contains two X's it is a male. Anatomical differences between males and hermaphrodites can be seen in Figure 2. Hermaphrodites can either self fertilize, or be fertilized by males, which becomes useful when performing crosses to generate progeny of a desired genotype. *C. elegans* are also ideal for these crosses because a single hermaphrodite can produce up to 350 offspring and their life-cycle is about 3 days (from egg to mature adult), which can be

result in time efficient experiments (The Characteristics of *C. elegans* that Make It Useful in Teaching, 1999).

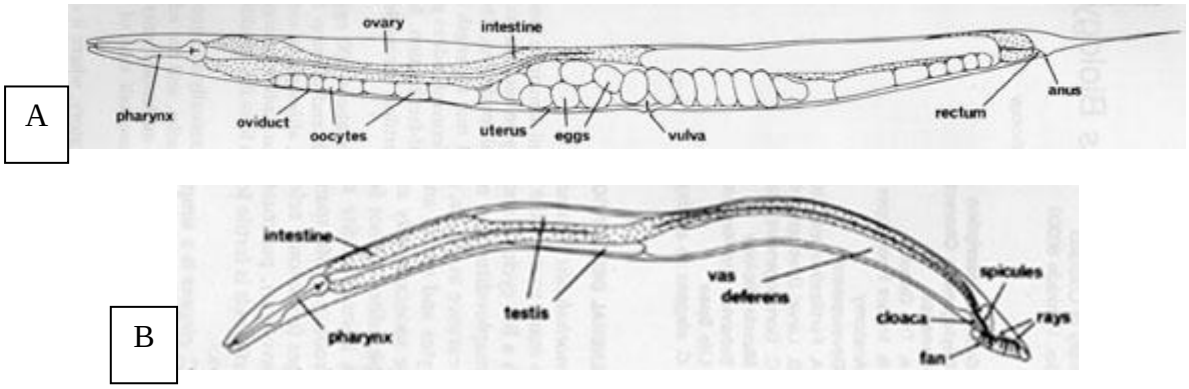


**Figure 2:** Anatomy of Hermaphrodite (A) and male (B) *C. elegans* taken from *The Characteristics of C. elegans that make it Useful in Teaching, 1999.*

Geneticists and cell biologists often use *C. elegans* as a model organism due to its physical feature which are convenient for such experiments. Having a genome size of about one hundred million base pairs, *C. elegans* have a genome approximately 30 times smaller than humans and can therefore be sequenced easier. In 1998 it was the first genome to be completely sequenced for a multi-cellular organism and since then has been useful in studying mutations and human diseases. *C. elegans* are also transparent, which allows for easy cell tracking and gene marking. Wild-type animals consistently contain 959 cells, which also make them convenient for cell fate tracking and experimentation (The Characteristics of *C. elegans* that Make It Useful in Teaching, 1999).

## 3. Function of the *mig-10* gene in Neuronal Migration and Axon Outgrowth

In *C. elegans* the *mig-10* gene plays an important role in embryonic migration. MIG-10 protein is a signaling molecule, an ortholog to lamellipodin in vertebrates (Quinn and Wadsworth, 2008). There are several neurons which are regulated by the *mig-10* gene: the Canal

Associated Neurons (CAN), Anterior Lateral Microtubule cells (ALM) and Hermaphrodite Specific Neurons (HSN) (Manser et al., 1997). CAN and ALM are known to migrate in an anterior to posterior direction, while HSN is known to migrate in the opposite direction. All three neurons however migrate to a position which is located at approximately half the length of the worm. This migration is shortened in both directions anterior to posterior for CAN and ALM, posterior to anterior for HSN, due to mutations in the *mig-10* gene which shows that *mig-10* is more likely to be involved in neuronal migration, rather than directional signaling (Manser and Wood, 1990). It has also been found that an over expression of MIG-10 in the absence of guidance cues can result in multiple misguided processes. These data suggest that MIG-10 is an outgrowth promoter (Quinn and Wadsworth, 2008).

The *mig-10* gene has also been found to be an essential factor in the development and migration of the excretory canal (Manser and Wood, 1990). The excretory cell of *C. elegans* is made up of a complex network of tubular epithelia forming an excretory canal which is located on the ventral side of the *C. elegans* near the developing pharynx (Buechner, 2002). The tubular formation of the canal is mediated through interactions between specific receptors on the membrane and the extracellular environment. Development of the excretory canal uses some of the same cues and mechanisms as neural migration and outgrowth which allows it to be used as a model when looking at the effect of the *mig-10* gene on its development (Buechner, 2002). A mutation in *mig-10* results in a shortened excretory canal.

## 4. The mechanism of MIG-10 Function

MIG-10 is a cytoplasmic adaptor protein that functions downstream from guidance cues UNC-6/Netrin and SLT-1/Slit (Quinn et al. 2006). Netrin is a guidance cue that is involved in

the attraction and repulsion of axons by binding to receptor DCC and UNC5. Slit is a protein that is secreted and acts as a guidance cue by acting as a repellent towards the growth cone. In the *C. elegans* embryo, Netrin is expressed ventrally, while Slit is expressed dorsally (Quinn et al. 2006). Normally, in response to these signals, AVM axons migrate ventrally. When MIG-10 is expressed in the absence of UNC-6 or SLT-1 some axons will migrate laterally rather than ventrally, and when both UNC-6 and SLT-1 are missing, the axons fail to migrate ventrally at all and form lateral axons. Over expression of MIG-10 in the absence of both guidance cues has resulted in AVM and PVM axons migrating in different areas or directions than normal, resulting in the display of a multipolar phenotype, but when either UNC-6 or SLT-1 is expressed the multipolar phenotype is suppressed and replaced with a monopolar phenotype. These studies imply that MIG-10 is an axon outgrowth promoter with no directional response, however in the presence of UNC-6 or SLT-1 a directional path guides axon outgrowth, which can also be enhanced by over expression of MIG-10 (Quinn et al., 2006). MIG-10 has been found to be a regulator for actin polymerization to promote filopodia and lamellipodin through interaction with UNC-34, as well as being an asymmetrically localized effector for Rac and PtdIns(3,4)P2 (Quinn and Wadsworth, 2008). It is also believed that MIG-10 also signals through other actin polymerization regulators because a loss of MIG-10 function causes a more drastic defect than loss of UNC-34 (Quinn and Wadsworth, 2008).

The *mig-10(mp0920)* mutation was isolated in 2009 by Jo-Ellen Sullivan and An Zacharia as a part of their major qualifying project (Sullivan and Zacharia, 2009). It was isolated by performing an EMS mutagenesis to induce point mutations in the *C. elegans* offspring. A simple screen was then performed that screened for mutations to the *mig-10* gene, which was done by screening for a phenotype displaying a truncated excretory canal. They also used

complementation tests to ensure that the mutation they had isolated was in fact located in the *mig-10* gene. Because the truncation of the *mp0920* allele was weaker than that of the null mutant *mig-10(ct41)* it is believed to be a missense mutation rather than a null mutation.

## 5. Project Objectives

The goal of this project was to characterize the phenotype of the *mig-10(mp0920) mutation* obtained from the work done by Sullivan and Zacharia (2009). This characterization was done by measuring migration of AVM and ALM neurons in mutant organisms and comparing the data to that of wild-type organisms. The location of the mutation and its effects on the gene was determined using PCR to determine sequence changes in the DNA of the *mig-10* locus. This can later be used to determine how the mutations affect translation of the DNA and any amino acid residue changes that may occur. Any possible residue change could affect the MIG-10 protein and potentially change its properties, resulting in phenotype changes in the animals. The data collected and analyzed in this project will be beneficial to the understanding of how changes in the MIG-10 protein domain affect its function. This could later lead to a better understanding of the MIG-10 homolog in humans.

## Methods

## 1. PCR

### 1.1 Worm Lysis

2.5µl of lysis buffer (50mM KCL, 10mM Tris (pH 8.3), 2.5 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20, 0.01% gelatin),which contained 60µg/mL proteinase K enzyme, was added to the cap of a PCR tube. Approximately ten worms were then added to the buffer in the caps using a platinum wire worm pick. Tubes containing the buffer and the worms were then centrifuged for 5 seconds to ensure the entire solution was at the bottom of the PCR tube. The tubes were then stored in a -80$^{o}$C freezer for at least 10 minutes, and could be stored for as long as necessary at -80$^{o}$C. Tubes were heated to 60$^{o}$C for 1 hour to activate the enzyme followed by 95$^{o}$C for 15 minutes to denature the Proteinase K.

### 1.2 PCR Cocktail Preparation

A PCR cocktail was prepared which was used for DNA amplification. This cocktail was made up of 2.5µl dNTP mix (mixed stock of 2.5mM of each dNTP), 2.5µl 25mM $MgCl_2$, 2.5µl Expand Long Template PCR buffer, 0.5µl Taq polymerase (5 Units/mL stock) and 9.5µl $dH_2O$ for each sample to be amplified. 2.5µl of each primer necessary primer was also added to their respective samples. The primers used for positive controls in the *mig-10(mp0920)* worms were mig10-wt1 (5'TGTTTGAATTTTCAGAATCCGC3') and mig10-wt2 (5'TGTTTCTTCTCACAATCCAACC3').

## 1.3 Primer Development for DNA Sequencing

Using Primer Quest from IDT DNA primers were designed that would be used for the sequencing of the coding region of the *mig-10* gene. The primers that were developed are displayed in table 1.

| Primer | Sequence |
|---|---|
| F1 | 5'AAATGTATCACGATCGACGGCGGA3' |
| R1 | 5'AACCAGTTGGAGCTCAGGAAGGAA3' |
| F2 | 5'AGGCAGTCCTAATTTGCCAGCTTC3' |
| R2 | 5'ATGTAACATTCGCGCGTTCAAGGG3' |
| F3 | 5'TGGACAGGTTAACTCCACCCACAA3' |
| R3 | 5'AGGACGAGAGATGAAGGCGTACTT3' |
| F4 | 5'TGCCTACCTACACGCTGGCAAATA3' |
| R4 | 5'TGGCCTCCCTATTAGACTTGCACT3' |
| F5 | 5'AGTATTGCAAGCGGGAAGACCATC3' |
| R5 | 5'TGTGTCAAGGCGATGTGAGTTGGT3' |
| F6 | 5'TCGAGCATTGGTTCGTCCTCAGAA3' |
| R6 | 5'GCTGTTAGTTCAGATCAGTGGCAG3' |
| F8 | 5'ACGCCTCCTCTCTTTATGTTCTCC3' |
| R8 | 5'GGTGTTTCGGACCGATTTCAAGCA3' |
| F9 | 5'GGCCTTGCATCACTTTGCTCTACT3' |
| R9 | 5'TGGGAGTCAAGTGTTTACGGACCA3' |

**Table 1:** Sequencing Primers

## 1.4 PCR Amplification

Once the PCR cocktail and proper primers were added to the samples the tubes were put in a thermal cycler and run through a reaction cycle. The amplification was started with an initial denaturing at 94$^{o}$C for 10 minutes which was used to disrupt hydrogen bonds in the DNA and yield single strands of DNA. The amplification cycle was then started and repeated 35 times. This cycle included 30 seconds of denaturing at 94$^{o}$C, 1 minute of annealing at 58$^{o}$ and 1 minute of primer extension at 72$^{o}$C. After 35 cycles, a final primer extension was performed at 72$^{o}$C for 10 minutes.

### 1.5 **Gel Electrophoresis**

The samples were electrophoresed in a 1.5% agarose gel (0.6g agarose/40mL 1xTBE). Hyperladder I was used as a marker ladder and the samples were marked using a loading buffer along with syber green. The gel was run at 120v for 1 hour.

## 2. Cross for *mig-10(mp0920); bgIs312; flp-20: GFP*

### 2.1 **him-5; mig-10(mp0920);flp-20::GFP**

In order to obtain a desired final strain containing the *mig-10(mp0920)* mutation, the *bgIs312* marker and an flp-20::GPF marker marking the ALM and AVM neurons a series of crosses had to be performed. The first cross that was made involved crossing N2 males with L4 hermaphrodites containing the *flp-20:GFP* marker. Offspring from this cross were homozygous for the *flp-20:GFP* marker. Males from this cross were then crossed with L$ hermaphrodites that were heterozygous for the *mig-10(mp0920)* mutation as well as the excretory cell marker bgIs312 . From these plates L4 hermaphrodite worms that expressed a phenotype containing the *flp-20:GFP* marker as well as a truncated EC were singled to separate plates and self crossed to determine homogeny. The plates that expressed the desired markers and phenotype were self crossed again and checked until the strain appeared 100% homozygous for the *mig-10(mp0920)* mutation and containing the *bgIs312* and *flp-20:GFP* markers. This cross can be seen below in figure 3.

Cross for *mig-10(mp0920);bgIs312; flp-20:GFP*

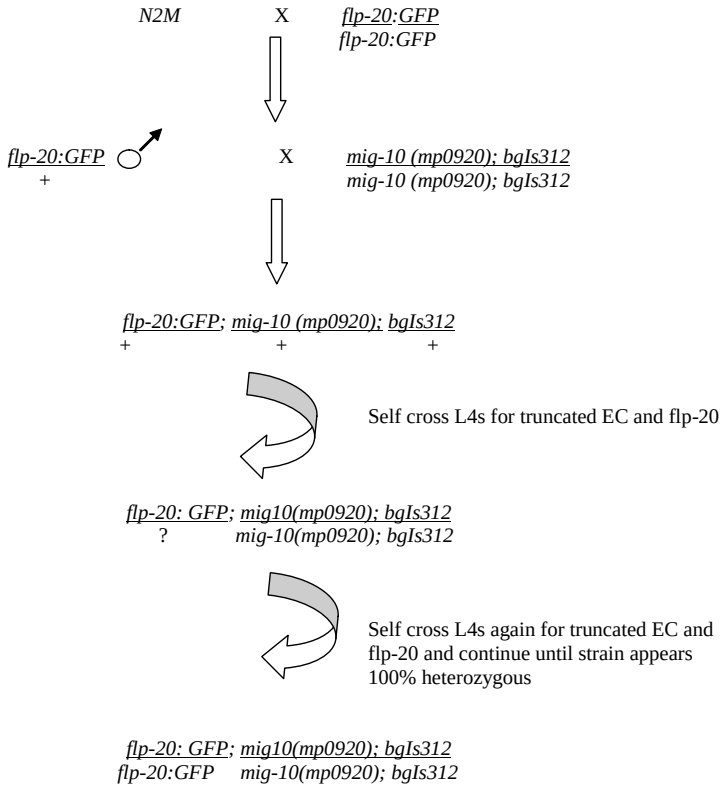


**Figure 3: cross** performed to obtain *mig-10(mp0920);bgIs312;flp-20:GFP* strain

## 3. Worm Quantitation

### 3.1 Slide Preparation

Slides were prepared by heating 2mL of agarose, in a test tube, by placing the tube in a beaker containing water and heating it to a boil. Once the agarose was melted 20µl of 1M sodium azide was added. Using a glass pipette one drop of agarose containing sodium azide was placed on a glass slide and immediately covered with a second slide to create an agar pad between slides.

## 3.2 Adding worms to slide

### 3.2.1 Picking worms to slide

Worms were picked into 4µl of M9 buffer was on the agar pad on the slide. A cover slip was then placed on top of the worms and the agar pad.

### 3.2.2 Washing worms from plate

Alternatively, worms were washed from the plate using M9 buffer. This was done by adding M9 buffer to the worm plate using a glass pipette and then swirling the plate so the worms were washed from the surface of the plate and suspended in the buffer. The buffer containing the worms was then removed from the plate using the same glass pipette and put in a 1mL eppindorf tube. Approximately 4µl of the buffer with the suspended worms were added to the slide containing the agar pad and covered with a cover slip.

### 3.2.3 Measuring cell migration

Using a fluorescent compound microscope with a camera the position of ALM and AVM cells with respect to the vulva were measured. This was done using the IpLab 3.7 program. The distance, in pixels, from the vulva to the center of the grinder of the pharynx was measured using the measure length tool; this measurement was used to normalize the data for different sized worms. It was important to follow along the gut when measuring. The distance from the vulva to the ALM cells as well as the AVM cell were then measured using the same method.

The length of the posterior and anterior excretory cell projection were also measured using a similar method. The posterior cell projection was measured from the cell body to the posterior end of the projection and normalized using the total length of the worm. The anterior projection was measured from the cell body to the anterior end of the projection and normalized using the distance from the center of the grinder of the pharynx to the nose of the animal.

## Results

In order to properly characterize the *mig-10 (mp0920)* mutation it was important to examine both the genotype and the phenotype of the worms containing the mutation. The genotype of the worms was examined by sequencing exons from the *mig-10* coding region in *mig-10 (mp0920)* mutant animals. These results were compared with the known *mig-10* wild type coding region from Wormbase. Characterizing the phenotype of the mutation involved measuring the excretory cell projection lengths (anterior and posterior) as well as the migration distances of the ALM and AVM neurons and comparing them with data collected from wild type and null mutants *mig-10 (ct41)*.

### 1. Sequencing of the *mig-10* coding region in *mig-10(mp0920)* mutants

The *mig-10* gene is located on the third chromosome of the *C. elegans* genome. The gene encodes three transcripts; the longest, *mig-10c* is 26,725 base pairs long with 2,340 coding nucleotides (Figure 4). Part of the characterization of the *mig-10(mp0920)* mutant involved sequencing the coding regions of the *mig-10* gene, using PCR to amplify the DNA, and comparing it to the known sequence of the wild type. This was done to determine where any point mutations in the gene might be located. Coding regions of the gene were of particular interest, because a mutation in the coding region would lead to a change in the MIG-10 protein domain. Four exons were sequenced, and were found to be wild type (Figure 4).

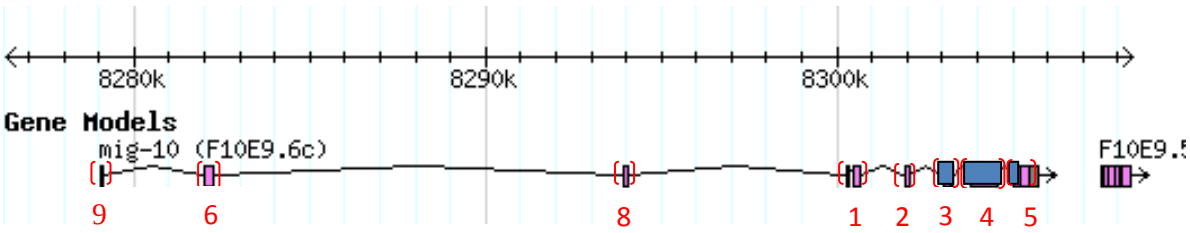


**Figure 4:** *mig-10c* Transcript Picture Including Primer Locations

In this figure the coding regions of the largest transcript of the *mig-10* gene, *mig-10c,* can be seen. The coding regions that are displayed as blue boxes are coding regions that were sequenced and were found to be wild type. Pink boxes represent coding regions that have not yet been sequenced. The red brackets and numbers annotate which primer pairs were designed to amplify each specific coding region The full genomic sequence of the *mig-10* gene, including primer locations, can be found in appendix A. Modified from Wormbase (wormbase.org, 2010).

## 2. *mig-10(mp0920)* phenotype quantitation

The phenotype of the *mig-10(mp0920)* mutant was characterized by determining the location of the ALM and AVM neurons, as well as the length of the anterior and posterior excretory cell projections. Measurements were made relative to fixed landmarks on the worm. To adjust for the size of each worm segments of the worms were used to normalize the lengths and allow for accurate comparison between strains of worms without the actual size of the worm being a factor. After normalizing the values of the migration distances, data from the *mig-10(mp0920)* mutant strain was compared to that of the wild type strain and a null mutant strain, *mig-10(ct41).* This analysis was done using analysis of variance (ANOVA) followed by post hoc tests. From these tests it was determined whether the migration distances were significantly different from the wild type or the null mutant.

## 2.1 Excretory Cell projection

MIG-10 plays an important role in the outgrowth of the excretory canal in *C. elegans* and because of this it was essential to determine the effect of the *mig-10(mp0920)* mutation on the length of the excretory cell projections, in both the posterior and anterior directions. The normalized data from the *mig-10(mp0920)* worms was compared with that of the wild type and the null mutant *mig-10(ct41)* to determine whether there was a significant difference between the strains. A schematic showing the measurements that were done for the excretory cell projections can be seen in figure 5.

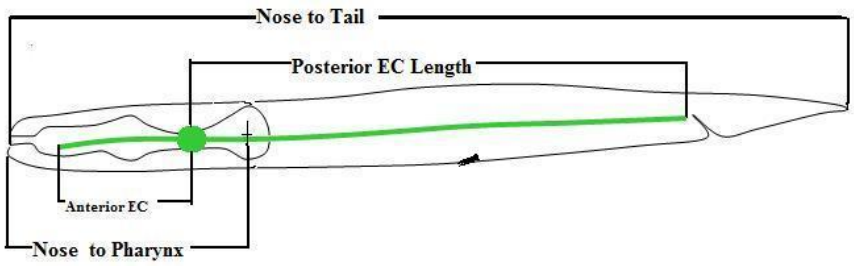


**Figure 5:** Measurements made to find normalized anterior and posterior EC lengths

### 2.1.1 Anterior Excretory Cell Projection

The anterior cell projection was measured from the cell body to the anterior end of the excretory canal and was normalized using the distance from the nose of the worm to its grinder, located in the pharynx, the measurement can be seen in figure 5. Statistical analysis showed that there was a significant difference in anterior cell projection between the *mig-10(mp0920)* mutant and the wild type ($p<.001$), but not between the *mig-10(mp0920)* mutant and the *mig-10(ct41)* null mutant ($p=.373$**).**Thus, *mig-10(mp0920)* is indistinguishable from the null mutant for this aspect of the phenotype. A bar graph displaying the mean normalized lengths of the anterior excretory cell projection is displayed in figure 6.

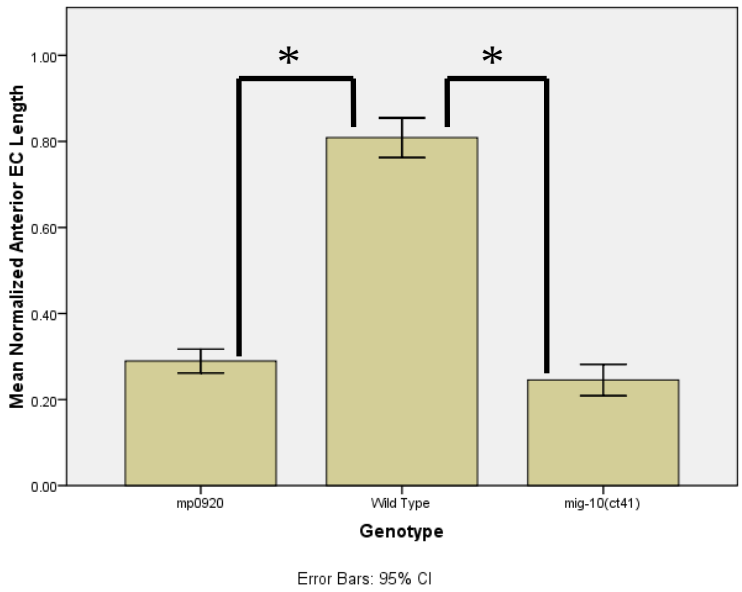


**Figure 6:** Mean Normalized Lengths of Anterior Excretory Projection

* annotates that there was a significant difference between phenotypes ($p<.001$) according to Bonferroni post hoc analysis.

### 2.1.2 Posterior Excretory Cell Projection

The posterior cell projection was measured from the cell body to the posterior end of the excretory canal and was normalized using the length of the worm from nose to tail (figure 5). Statistical analysis showed that there was a significant difference in exterior cell projection between all three of the strains ($p<.001$). Figure 7 shows a bar graph displaying the normalized mean lengths of the posterior excretory canal between strains. Therefore, *mig-10(mp0920)* is intermediate between the null mutant and the wild type in this aspect of the phenotype.

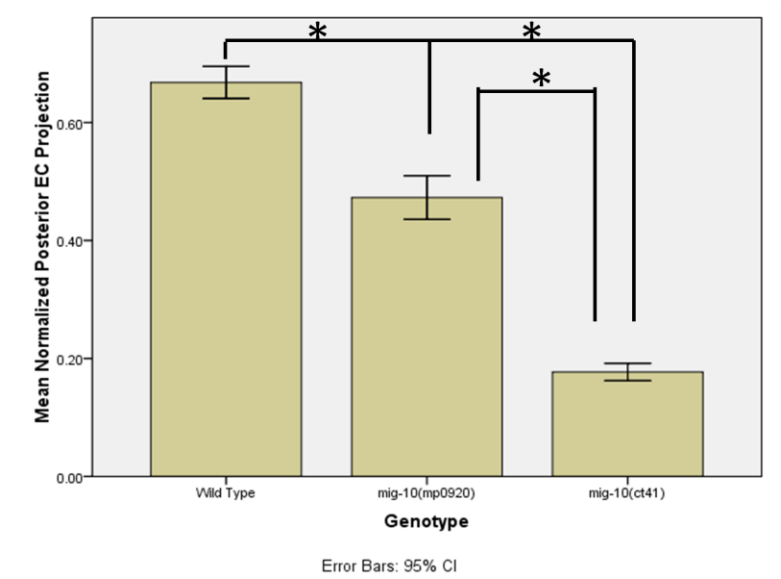


**Figure 7:** Mean Normalized Lengths of Posterior Excretory Projection

* annotates that there was a significant difference ($p<.001$) between phenotypes according to Bonferroni post hoc analysis.

## 2.2 Neuron Migration

MIG-10 also plays an important role in neuron migration in *C. elegans* which is why it was important to determine the effect the *mig-10(mp0920)* mutation has on the migration of neurons AVM and ALM. The normalized data from the *mig-10(mp0920)* worms was compared with that of the wild type and the null mutant, *mig-10(ct41)*, to determine whether there was a significant difference between the strains. Figure 8 shows the measurements that were performed to obtain normalized neuron migration distances.

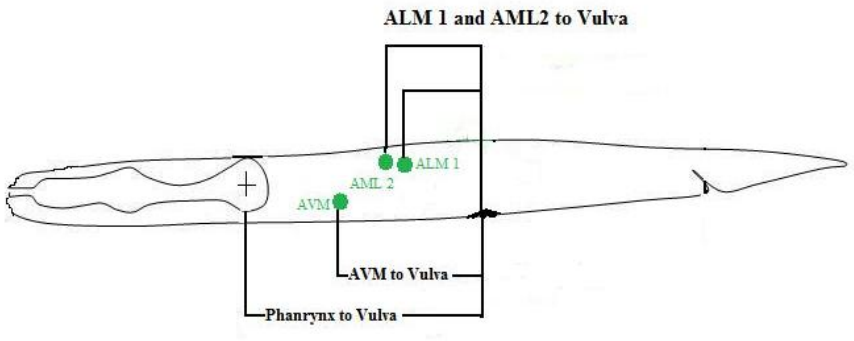


**Figure 8:** Measurements made to find normalized neuron migration distances.

### 2.2.1 ALM 1 Migration

Migration of ALM 1 was determined by measuring the distance from the vulva of the worm to the cell and was normalized using the length from the grinder located in the pharynx of the worm to the vulva (figure 8). Because there are two ALM neurons (one on each side of the animal) ALM1 was defined as the cell the shortest distance from the vulva while ALM2 was further away. Thus, ALM1 was defined as the cell that had migrated furthest from its initial location. This was done to keep consistency in the data. A statistical analysis displayed that there was a significant difference in ALM1 migration distance between *mig-10 (mp0920)* and *mig-10(ct41)* null mutant (p=.035). There was not, however, a significant difference between *mig-10(mp0920)* and wild type (p=1.000) or between the wild type and the null mutant (p=0.051). Thus, *mig-10(mp0920)* is indistinguishable from the wild type in this aspect of the phenotype. Figure 9 displays a bar graph containing normalized ALM1 migration distances.

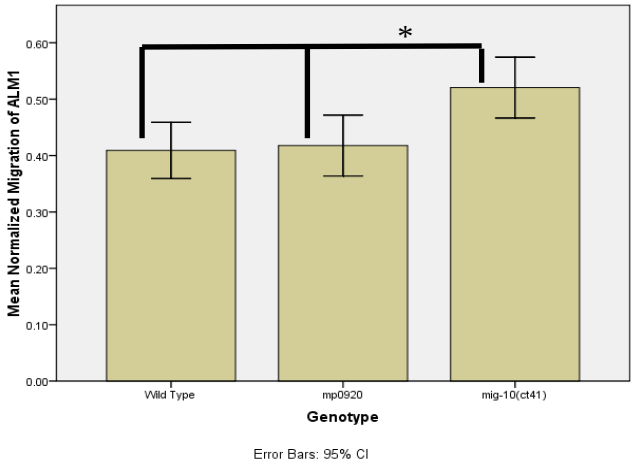


**Figure 9:** Mean Normalized ALM1 Migration Distances

* annotates that there was a significant difference (p=0.35) between phenotypes according to Bonferroni post hoc analysis.

### 2.2.2 ALM 2 Migration

Migration of ALM 2 was determined by measuring the distance from the vulva of the worm to the cell (ALM 2 was the ALM cell further from the vulva) and was normalized using the length from the grinder located in the pharynx of the worm to the vulva (figure 8). Statistical analysis of the data displayed that there was a significant difference between the *mig-10(mp0920)* mutant and wild type (p=.001), as well as between wild type and the null mutant, *mig-10(ct41),* (p=0.29). There was not, however, a significant difference between the *mig-10(mp0920)* mutant and the null mutant (p=1.000).Thus *mig-10(mp0920)* is indistinguishable from the null mutant, *mig-10(ct41),* in this aspect of the phenotype. Figure 10 shows a bar graph that contains the normalized ALM2 migration distances.

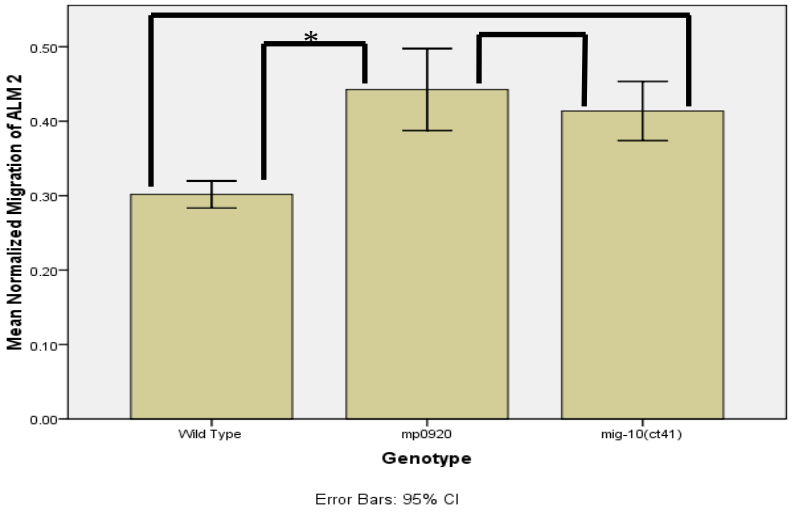


**Figure 10:** Mean Normalized ALM2 Migration Distances

* annotates that there was a significant difference (p=.001) between phenotypes according to Bonferroni post hoc analysis.

### 2.2.3 AVM Migration

The migration of AVM was determined by measuring the distance from the vulva of the worm to the cell and was normalized using the length from the grinder located in the pharynx of the worm to the vulva (figure 8). Statistical analysis of the data displayed there was a significant difference AVM migration between *mig-10(mp0920)* and the null mutant, *mig-10(ct41)*, (p<.001) and between the null mutant and the wild type (p<.001), but not between the *mig-10(mp0920)* mutant and the wild type (p=.593). Thus, *mig-10(mp0920)* is indistinguishable from the wild type in this aspect of the phenotype. Figure 11 displays the normalized AVM migration distances.

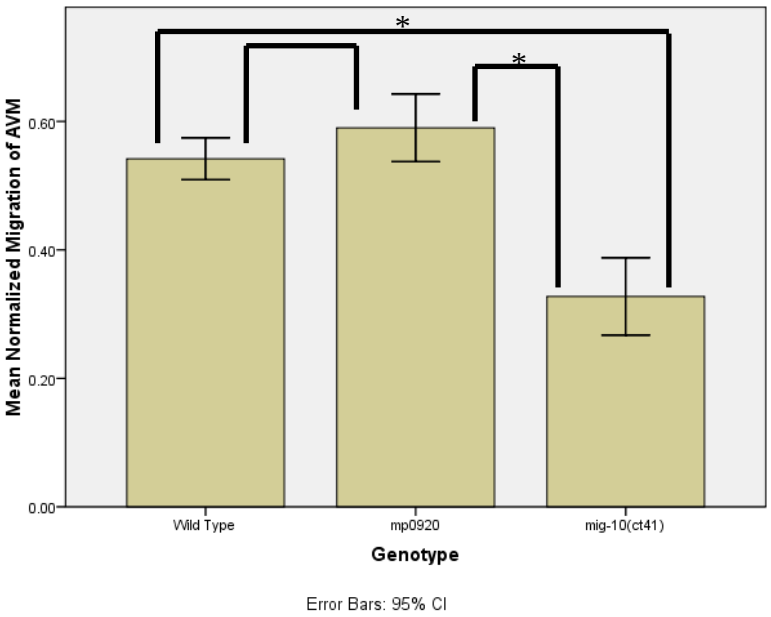


**Figure 11:** Mean Normalized AVM Migration Distances

In this figure a chart displaying the differences in normalized AVM migration distances is are displayed.
* annotates that there was a significant difference ($p<.001$) between phenotypes according to Bonferroni post hoc analysis.

## Discussion

The goals of this project were to characterize both the phenotype and genotype of the *mig-10(mp0920)* mutation in *C. elegans* in order to help define the MIG-10 protein domain and its effects on neuronal migration. This was done by measuring the excretory canal lengths as well as migrations distances of ALM and AVM neuronal cells and comparing them to data from wild type and a null mutant, *mig-10(ct41)*. Sequencing of the coding regions of the *mig-10* genome was also performed with the intention of locating a possible point mutation.

From the partial sequencing of the *mig-10* coding region that was performed, the exons that were sequenced were found to be wild type. This does not, however, mean that a mutation does not exist somewhere else in the *mig-10* gene. If the primers that have been designed for the sequencing of this gene are made to work properly the remaining coding regions of the *mig-10* gene in *mig-10(mp0920)* mutants could be sequenced and compared to the known wild type genome. From this it could be determined whether or not the mutation is located in the coding region of the gene and if so what its affect on the MIG-10 protein domain might be.

Characterization of the *mig-10(mp0920)* phenotype showed that the *mig-10(mp0920)* resembles null for the anterior projection and intermediate between the null and wild type for the posterior projection. These results can help us determine that the *mig-10(mp0920)* mutation has a significant effect on the length of the excretory canal, but is not as severe the null mutant, which suggests that the mutation may code for a change in the MIG-10 protein domain rather than stopping its production completely. The effect of the *mig-10(mp0920)* mutation on the ALM and AVM cells, however, was not as drastic. Data showed that the only significant difference in migration distance between the *mig-10(mp0920)* mutant and the wild type was for the migration

of ALM2, further suggesting that the *mig-10(mp0920)* mutation is unlikely to result in a stop codon in the coding region.

In conclusion the *mig-10(mp0920)* mutation appears to resemble an intermediate between wild type and null for the posterior cell projection, but appears to be null in the anterior projection. The mutation also appears to have an effect on the migration of ALM neurons, but not AVM, this could be due to migrations occurring during different stages of development. This means the mutation occurring in *mig-10(mp0920)* could affect the MIG-10 protein domain in a way that only hinders the excretory cell and ALM migration. Another possibility, if the mutation is not found to be in the protein domain, is that the mutation one that lowers the amount of MIG-10 protein produced, such as a splicing mutation. Future work could include comparing the *mig-10(mp0920)* phenotype with other known *mig-10* mutations to evaluate the different effects the mutations have on cell migration and excretory canal outgrowth. It would also be beneficial to continue with the sequencing of the coding regions of the *mig-10* gene in *mig-10(mp0920)* mutants and aligning it with the known sequence to find were any mutations may exist. A better understanding of the MIG-10 protein domain will lead to advancements in our knowledge of the human homolog lamellipodin.

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## Appendix A: *mig-10c* Sequence containing primer locations

F10E9.6c (1-26725) (unspliced + UTR - 26725 bp)

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atttt

Primers:

F1: 5’AAATGTATCACGATCGACGGCGGA3’

R1: 5’AACCAGTTGGAGCTCAGGAAGGAA3’

Rev Comp: TTCCTTCCTGAGCTCCAACTGGTT

836 bp

F2: 5’AGGCAGTCCTAATTTGCCAGCTTC3’

R2:5’ ATGTAACATTCGCGCGTTCAAGGG 3’

Rev Comp: CCCTTGAACGCGCGAATGTTACAT

1097 bp

F3: 5’TGGACAGGTTAACTCCACCCACAA3’

R3: 5’ AGGACGAGAGATGAAGGCGTACTT 3’

Rev Comp: AAGTACGCCTTCATCTCTCGTCCT

1020 bp

F4: 5’TGCCTACCTACACGCTGGCAAATA3’

R4: 5’AGTGCAAGTCTAATAGGGAGGCCA 3’

Rev Comp: tggcctccctattagacttgcact

1206bp

F5:5′AGTATTGCAAGCGGGAAGACCATC3′
R5:5′ TGTGTCAAGGCGATGTGAGTTGGT 3′
Rev Comp:ACCAACTCACATCGCCTTGACACA

709bp

F6: 5′TCGAGCATTGGTTCGTCCTCAGAA3′
R7: 5′GCTGTTAGTTCAGATCAGTGGCAG3′
Rev comp: CTGCCACTGATCTGAACTAACAGC

1026bp

F8: 5′ACGCCTCCTCTCTTTATGTTCTCC3′
R8: 5′GGTGTTTCGGACCGATTTCAAGCA3′
Rev comp: TGCTTGAAATCGGTCCGAAACACC

508bp

F9: 5′GGCCTTGCATCACTTTGCTCTACT3′
R9: 5′TGGGAGTCAAGTGTTTACGGACCA3′
Rev Comp: TGGTCCGTAAACACTTGACTCCCA

906bp

## Appendix B: Sequenced Coding Regions

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GATCCTTGTGTTTGCATGAATCCTCCCCCCCCCTGACGGACCCCGTTTATGATTTATCTGCAAGATCTTTAGAAAGCT
CCTGGCTTGAACGATATTAGACGTTGCCAATGATACCTTGTAGTGCATGGC

## Appendix C: Sequence Alignment Map

mig-10c.str from 1 to 29329

**F3**
**(23905...24169)**

**R3**
**(24141...23847)**

**R3 mismatch**
**(24135...24135)**

**R3 mismatch**
**(24126...24126)**

**F4**
**(24869...25613)**

**R4**
**(25562...24828)**

**F5**
**(26126...26309)**

**R5**
**(26309...26074)**

**R5**
**(26074...26310)**

**R5 mismatch**
**(26308...26306)**

**R5 mismatch**
**(26303...26293)**

**R5 mismatch**
**(26290...26284)**

**R5 mismatch**
**(26281...26281)**

**R5 mismatch**
**(26278...26278)**

**R5 mismatch**
**(26275...26275)**

**R5 mismatch**
**(26263...26263)**

## Appendix D: Post Hoc Analysis

### Multiple Comparisons

Dependent Variable: Normalized Anterior Excretory Projection

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | -.5191* | .02917 | .000 |
| | | mig-10(ct41) | .0441 | .02840 | .373 |
| | Wild Type | mp0920 | .5191* | .02917 | .000 |
| | | mig-10(ct41) | .5632* | .02578 | .000 |
| | mig-10(ct41) | mp0920 | -.0441 | .02840 | .373 |
| | | Wild Type | -.5632* | .02578 | .000 |

Based on observed means.
The error term is Mean Square (Error) = .010.

*. The mean difference is significant at the .05 level.

**Table 1:** Bonferroni Analysis of Anterior Excretory Projection
In this table the comparison in normalized lengths of the anterior cell projection is shown. A significance of less than 0.5 determined that there was a significant difference between the strains of the worms. This table shows that there was a significant difference between the wild type and both the mp0920 mutant as well as the null mutant (ct41), but not a significant difference between the null mutant and mp0920.

| | Genotype | N | Subset 1 | Subset 2 |
|---|---|---|---|---|
| Tukey B[a,,b,,c] | mig-10(ct41) | 32 | .2454 | |
| | mp0920 | 20 | .2896 | |
| | Wild Type | 28 | | .8087 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .010.

a. Uses Harmonic Mean Sample Size = 25.649.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

| | Genotype | N | Subset 1 | Subset 2 |
|---|---|---|---|---|
| Tukey B[a,,b,,c] | mig-10(ct41) | 32 | .2454 | |
| | mp0920 | 20 | .2896 | |
| | Wild Type | 28 | | .8087 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .010.

a. Uses Harmonic Mean Sample Size = 25.649.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

**Table 2:** Tukey Analysis of Anterior Cell Projection

This table displays the normalized mean anterior cell projection lengths of the three strains of worms that were compared. Using this method strains that had significantly different means were separated into different subsets, while strains that were not significantly different remained in the same subset. In this table *mig-10(mp0920)* and *mig-10 (ct41)* were grouped into the same subset while the wild type was put into a different one, showing that mp0920 and ct41 both had significantly shorter migration distances than the wild type but were not significantly different from each other.

**Multiple Comparisons**

Dependent Variable: Normalized Posterior EC Projection

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | -.1952* | .01928 | .000 |
| | | mig-10(ct41) | .2958* | .01865 | .000 |
| | Wild Type | mp0920 | .1952* | .01928 | .000 |
| | | mig-10(ct41) | .4910* | .01899 | .000 |
| | mig-10(ct41) | mp0920 | -.2958* | .01865 | .000 |
| | | Wild Type | -.4910* | .01899 | .000 |

Based on observed means.
The error term is Mean Square (Error) = .005.

*. The mean difference is significant at the .05 level.

**Table 3:** Bonferroni Analysis of Posterior Excretory Projection
In this table the comparison in normalized lengths of the posterior cell projection is shown. A significance of less than 0.5 determined that there was a significant difference between the strains of the worms. This table shows that there was a significant difference between the all three of the strains that were compared.

| | | | Subset | | |
|---|---|---|---|---|---|
| | Genotype | N | 1 | 2 | 3 |
| Tukey B[a,,b,,c] | mig-10(ct41) | 32 | .1770 | | |
| | mp0920 | 30 | | .4728 | |
| | Wild Type | 28 | | | .6680 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .005.

a. Uses Harmonic Mean Sample Size = 29.911.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

**Table 4:** Tukey Analysis of Posterior Cell Projection
This table displays the normalized mean posterior cell projection lengths of the three strains of worms that were compared. Using this method strains that had significantly different means were separated into different subsets. In this three different subsets were formed, one for each of the phenotypes measured. From this it can be determined that there was a significant difference in posterior excretory canal length between each of the three strains.

Multiple Comparisons

Dependent Variable: Normalized ALM1 migration distance

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | .0087 | .03855 | 1.000 |
| | | mig-10(ct41) | -.1029* | .03946 | .035 |
| | Wild Type | mp0920 | -.0087 | .03855 | 1.000 |
| | | mig-10(ct41) | -.1115 | .04530 | .051 |

| | | | | | |
|---|---|---|---|---|---|
| | mig-10(ct41) | mp0920 | .1029* | .03946 | .035 |
| | | Wild Type | .1115 | .04530 | .051 |

Based on observed means.

The error term is Mean Square (Error) = .015.

*. The mean difference is significant at the .05 level.

**Table 5:** Bonferroni Analysis of ALM1 migration

In this table the comparison in normalized ALM1 migration distances is shown. A significance of less than 0.5 determined that there was a significant difference between the strains of the worms. This table shows that the only significant difference in migration distance was between the wild type and the null mutant.

| | | | Subset | |
|---|---|---|---|---|
| | Genotype | N | 1 | 2 |
| Tukey B[a,,b,,c] | Wild Type | 15 | .4091 | |
| | mp0920 | 30 | .4177 | |
| | mig-10(ct41) | 14 | | .5206 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square (Error) = .015.

a. Uses Harmonic Mean Sample Size = 17.500.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

**Table 6:** Tukey Analysis of ALM1 migration

This table displays the normalized mean ALM1 migration distances of the three strains of worms that were compared. Using this method strains that had significantly different means were separated into different subsets, while strains that were not significantly different remained in the same subset. In this table the wild type and *mig-10(mp0920)* were grouped into the same subset while the null mutant was put into a different one, showing that mp0920 and wild type both had significantly longer migration distances than the null mutant but were not significantly different from each other.

Multiple Comparisons

Dependent Variable:ALM2norm

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | .1409* | .03552 | .001 |
| | | mig-10(ct41) | .0288 | .03635 | 1.000 |
| | Wild Type | mp0920 | -.1409* | .03552 | .001 |
| | | mig-10(ct41) | -.1121* | .04174 | .029 |
| | mig-10(ct41) | mp0920 | -.0288 | .03635 | 1.000 |
| | | Wild Type | .1121* | .04174 | .029 |

Based on observed means.

The error term is Mean Square (Error) = .013.

*. The mean difference is significant at the .05 level.

**Table 7:** Bonferroni Analysis of ALM2 migration

In this table the comparison in normalized ALM2 migration distances is shown. A significance of less than 0.5 determined that there was a significant difference between the strains of the worms. This table shows that there was a significant difference in migration distance was between the wild type and *mig-10 (mp0920)* as well as between the wild type and the null mutant.

| | Genotype | N | Subset 1 | Subset 2 |
|---|---|---|---|---|
| Tukey B[a,,b,,c] | Wild Type | 15 | .3016 | |
| | mig-10(ct41) | 14 | | .4137 |
| | mp0920 | 30 | | .4425 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .013.

a. Uses Harmonic Mean Sample Size = 17.500.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

**Table 8:** Tukey Analysis of ALM2 migration

This table displays the normalized mean ALM2 migration distances of the three strains of worms that were compared. Using this method strains that had significantly different means were separated into different subsets, while strains that were not significantly different remained in the same subset. In this table the wild type was placed in its own subset, while *mig-10(mp0920)* and *mig-10(ct41)* were grouped into the same subset showing that mp0920 and the null mutant both had significantly shorter migration distances than the wild type but were not significantly different from each other.

**Multiple Comparisons**

Dependent Variable: Normalized AVM Migration Distance

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | .0482 | .03694 | .593 |
| | | mig-10(ct41) | .2626* | .03781 | .000 |
| | Wild Type | mp0920 | -.0482 | .03694 | .593 |
| | | mig-10(ct41) | .2144* | .04341 | .000 |
| | mig-10(ct41) | mp0920 | -.2626* | .03781 | .000 |
| | | Wild Type | -.2144* | .04341 | .000 |

Based on observed means.
The error term is Mean Square (Error) = .014.

**Multiple Comparisons**

Dependent Variable: Normalized AVM Migration Distance

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | .0482 | .03694 | .593 |
| | | mig-10(ct41) | .2626* | .03781 | .000 |
| | Wild Type | mp0920 | -.0482 | .03694 | .593 |
| | | mig-10(ct41) | .2144* | .04341 | .000 |
| | mig-10(ct41) | mp0920 | -.2626* | .03781 | .000 |
| | | Wild Type | -.2144* | .04341 | .000 |

Based on observed means.
The error term is Mean Square (Error) = .014.

*. The mean difference is significant at the .05 level.

**Table 9:** Bonferroni Analysis of AVM migration

In this table the comparison of normalized AVM migration distances is shown. A significance of less than 0.5 determined that there was a significant difference between the strains of the worms. This table shows that there was a significant difference in migration distance between *mig-10 (mp0920)* and the null mutant as well as between the wild type and the null mutant.

| | Genotype | N | Subset 1 | Subset 2 |
|---|---|---|---|---|
| Tukey B[a,,b,,c] | mig-10(ct41) | 14 | .3275 | |
| | Wild Type | 15 | | .5419 |
| | mp0920 | 30 | | .5901 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .014.

a. Uses Harmonic Mean Sample Size = 17.500.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

**Multiple Comparisons**

Dependent Variable: Normalized AVM Migration Distance

| | (I) Genotype | (J) Genotype | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|---|
| Bonferroni | mp0920 | Wild Type | .0482 | .03694 | .593 |
| | | mig-10(ct41) | .2626* | .03781 | .000 |
| | Wild Type | mp0920 | -.0482 | .03694 | .593 |
| | | mig-10(ct41) | .2144* | .04341 | .000 |
| | mig-10(ct41) | mp0920 | -.2626* | .03781 | .000 |
| | | Wild Type | -.2144* | .04341 | .000 |

Based on observed means.

The error term is Mean Square (Error) = .014.

c. Alpha = .05.

**Table 10:** Tukey Analysis of AVM migration

This table displays the normalized mean AVM migration distances of the three strains of worms that were compared. Using this method strains that had significantly different means were separated into different subsets, while strains that were not significantly different remained in the same subset. In this table the null mutant was placed in its own subset, while *mig-10(mp0920)* and the wild type were grouped into the same subset showing that mp0920 and the wild type both had significantly longer migration distances than the null mutant but were not significantly different from each other.